AD_____

Award Number: DAMD17-99-1-9135

TITLE: Characterization of Two C. elegans Homologuses of Oncogenic Inhibitor of Apoptosis Proteins (IAPs) and Identification of Interacting Genes

PRINCIPAL INVESTIGATOR: Andrew G. Fraser, Ph.D.

CONTRACTING ORGANIZATION: University of Cambridge
Cambridge CB2 1TS England

REPORT DATE: September 2001

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2001	Annual Summary (1 Aug 00 - 1 Aug 01)

4. TITLE AND SUBTITLE
Characterization of Two C. elegans Homologuses of Oncogenic Inhibitor of Apoptosis Proteins (IAPs) and Identification of Interacting Genes

5. FUNDING NUMBERS
DAMD17-99-1-9135

6. AUTHOR(S)
Andrew G. Fraser, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Cambridge
Cambridge CB2 1TS England

E-Mail: agf10@cam.ac.uk

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
7

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Annual Summary Report for Award Number DAMD17-99-1-9135

FAO : Ms. Judy Pawlus, U.S. Army Medical Research and Materiel Command (MCMR-RMI-S), 504 Scott Street, Fort Detrick, Maryland 21702-5012.

From: Andrew G. Fraser, Ph.D., Wellcome/CRUK Institute of Cancer and Developmental Biology, Tennis Court Road, Cambridge CB2 1QR, United Kingdom

Background and Current Research

Although there is now a phenomenal quantity of sequence data available, biological function has only been assigned to a small percentage of predicted genes in any metazoan. Understanding how genetic information relates to biological function at the level not only of a single gene but of an entire genome is thus a key problem. One approach is to analyse loss-of-function phenotypes of every predicted gene and thus attempt to draw both global and particular conclusions about the relationship between gene sequence, predicted protein product and function in the organism. This is the approach that I have been pursuing for the last 2 years in the nematode worm, *C. elegans*.

We have generated a reagent that uses RNA-mediated interference (RNAi) to individually inhibit ~90% of all ~19,000 predicted genes in the *C. elegans* genome simply by feeding dsRNA-expressing bacteria to worms. This technique is both efficient and technically easy, so a single person can analyse loss-of-function phenotypes of up to 1000 genes in a single day for minimal cost; in addition, the reagent can be used an indefinite number of times. In essence, most genetic screens in *C. elegans* that could hitherto be carried out using standard forward genetics can now be carried out using our RNAi reagent, which has the advantage that any detected phenotype is automatically related to gene sequence, obviating the need for positional cloning.

We have already analysed loss-of-function phenotypes of ~40% of all predicted genes in *C. elegans* by RNAi in wild-type worms and expect to have completed analysis of ~90% of genes by the end of 2001. This will be the first study to characterise the function of genes in a metazoan genome in such a systematic and comprehensive manner. Our analysis has increased the number of sequenced genes with known phenotypes by a factor of 5 and, in addition to identifying

new functions for many interesting individual genes, we have been able to draw global conclusions about general relationships between gene sequence and function — for example, we find that genes that only affect metazoan biology (e.g. neuromuscular system) are likely to encode proteins with a more complex domain architecture, and to be enriched for domains that are only encoded in metazoan genomes. In addition, comparison of RNAi phenotypes of genes on autosomes with those on the X-chromosome has revealed that far fewer essential functions are encoded by genes on the X chromosome. We have found this to be principally due to a major difference in the relationship between the predicted molecular function of a gene products and the role they play in the organism; for example, genes encoding components of signal transduction pathways on the X chromosome are over 20-fold less likely to play an essential role than those on autosomes. We attribute this change to the fact that males are hemizygous for the X chromosome, thus making any somatic mutation in an essential gene on X deleterious in males.

Analysis of signal transduction in *C. elegans* by genome-wide RNAi screens

Our analysis of RNAi phenotypes has thus far been restricted to the identification of genes with grossly observable loss-of-function phenotypes in wild-type animals (~12% of all genes) and to analysis by time-lapse videomicroscopy of genes with an embryonic lethal RNAi phenotype. These data will be very useful in themselves, both because they provide an excellent start-point for more directed research (e.g. studying all genes that have a sterile RNAi phenotype to understand their roles in the germline) and because they will permit detailed analysis of the relationship between gene sequence and function *in vivo*. However, it is possible to carry out more specific screens using the RNAi feeding library.

My principal research direction over the next few years, aside from the functional genomics outlined above, will be to exploit the RNAi feeding library to carry out multiple genome-wide RNAi screens to identify genes involved in specific signalling pathways (e.g. ras and TGF-β), many of which play key roles in human cancer. In particular, I am interested in genes that affect more than one pathway, as these may provide insight into the mechanisms of crosstalk between pathways. To this end, I am carrying out genome-wide RNAi screens to identify genes that enhance or suppress phenotypes resulting from alterations in key signalling pathways.

Identification of modulators of ras signalling in *C. elegans*

Ha-ras is a small GTPase that is required in many signalling pathways (e.g. PDGF, IGF-1 signalling) and for many biological processes (mitogenesis, senescence, suppression of apoptosis etc.) and is mutated in a large fraction of human tumours. The *C. elegans* homologue of Ha-ras, *let-60*, is required at multiple stages in development, ranging from maturation of germ-cells, to specification of vulval fate, to a requirement in olfaction. Weakly activating mutations in *let-60* cause ~60% of adult worms to have multiple vulvae (Muv phenotype) due to a conversion of hypodermal cells to a vulval fate. One can therefore use the RNAi feeding library to screen for genes that modulate ras signalling by identifying those that either suppress this Muv phenotype (these will give increased Muv worms after RNAi) or that enhance the Muv phenotype (these will have reduced Muv phenotypes by RNAi).

I have begun screening for genes that alter the multivulval phenotype caused by an activated allele of *let-60*. Screening of ~1000 genes (5% of the genome) has already identified (amongst others) three suppressors of ras signalling previously uncharacterised in the worm, but known to be involved in ras signalling in other eukaryotes. These genes are homologues of a DAG-kinase, of *S. cerevisiae* YAK1 and of a putative component of a nucleosome remodelling complex. That these genes have not been identified as ras modulators in the many similar forward genetic screens already carried out confirms the strength of RNAi-based screens.

This screen has not only identified genes that synergise with activated ras in the vulva, but also at other developmental stages and in other tissues. Through our previous work, the RNAi phenotype of every gene is already known in a wild-type background and thus any gene that has a different phenotype in the context of an activated allele of ras to that in wild-type in some way synergises with ras. I have a number of excellent candidates for such genes which I am currently characterising; these have phenotypes that range from lethality to sterility to uncoordinated behaviour.

Finally, in screening chromosomes I, II and X for RNAi phenotypes in wild-type worms, I identified 6 genes that had Muv RNAi phenotypes; these are clearly candidates for modulators of ras signalling in the vulva. One of these, *lin-31*, is a known target of ras signalling in the vulva, confirming that our methods recapitulate known results. We also identified Muv RNAi phenotypes for a component of a nucleosome remodelling complex, which is known to repress ras signalling in the vulva, and two genes (*par-3* and *cdc-42*) that play a role in cell polarity; we subsequently demonstrated that other genes (e.g. *par-1, par-2*) that affect embryonic polarity also alter ras signalling in the vulva, and are currently elucidating the mechanism. Finally, we identified two conserved but uncharacterised genes that are strongly Muv following RNAi; both of these are shown to be expressed uniquely in the vulval cells by *in situ* hybridisation, suggesting that they do indeed play a role in vulval fate. We are characterising these further.

I intend to extend these screens across the entire genome along with other similar screens to identify modulators of TGF-β and Wnt signalling pathways. Ultimately, I intend to place each ras modulator identified into the ras pathway in *C. elegans* to understand the mechanism by which they modulate ras signalling. Furthermore, I will characterise the signalling role of mammalian homologues of each identified gene, to test whether what I observe in *C. elegans* is applicable to mammals. This will principally be done by combining *in vitro* assays (e.g. two-hybrid interactions) and functional analysis in cell-lines.